Molecular Biology (7)

Transcription-Regulation

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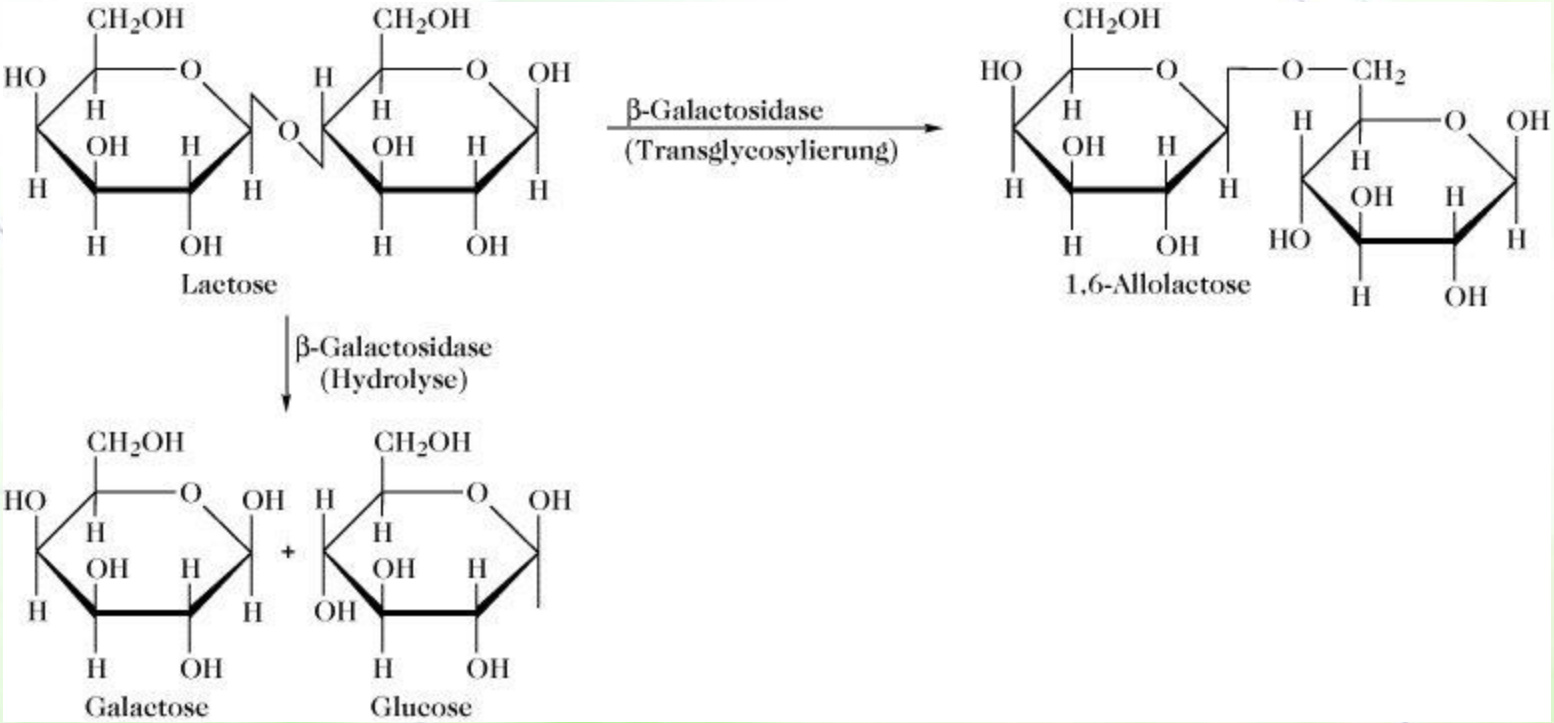
Regulation of transcription in prokaryotes

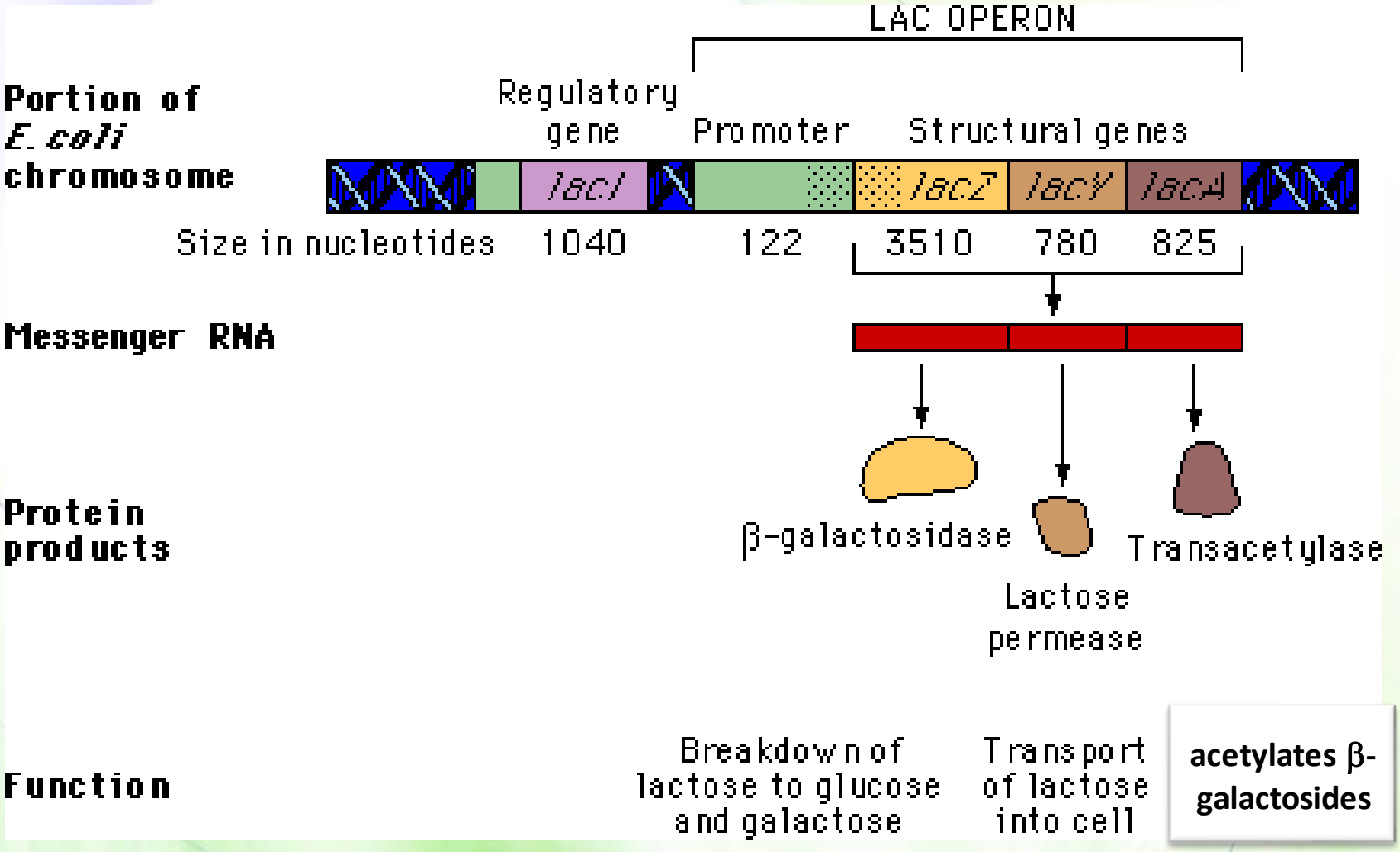
The lac operon

Metabolism of lactose

- In the 1950s, pioneering experiments were carried out by François Jacob and Jacques Monod who studied regulation of gene transcription in E. coli by analyzing the expression of enzymes involved in the metabolism of lactose.

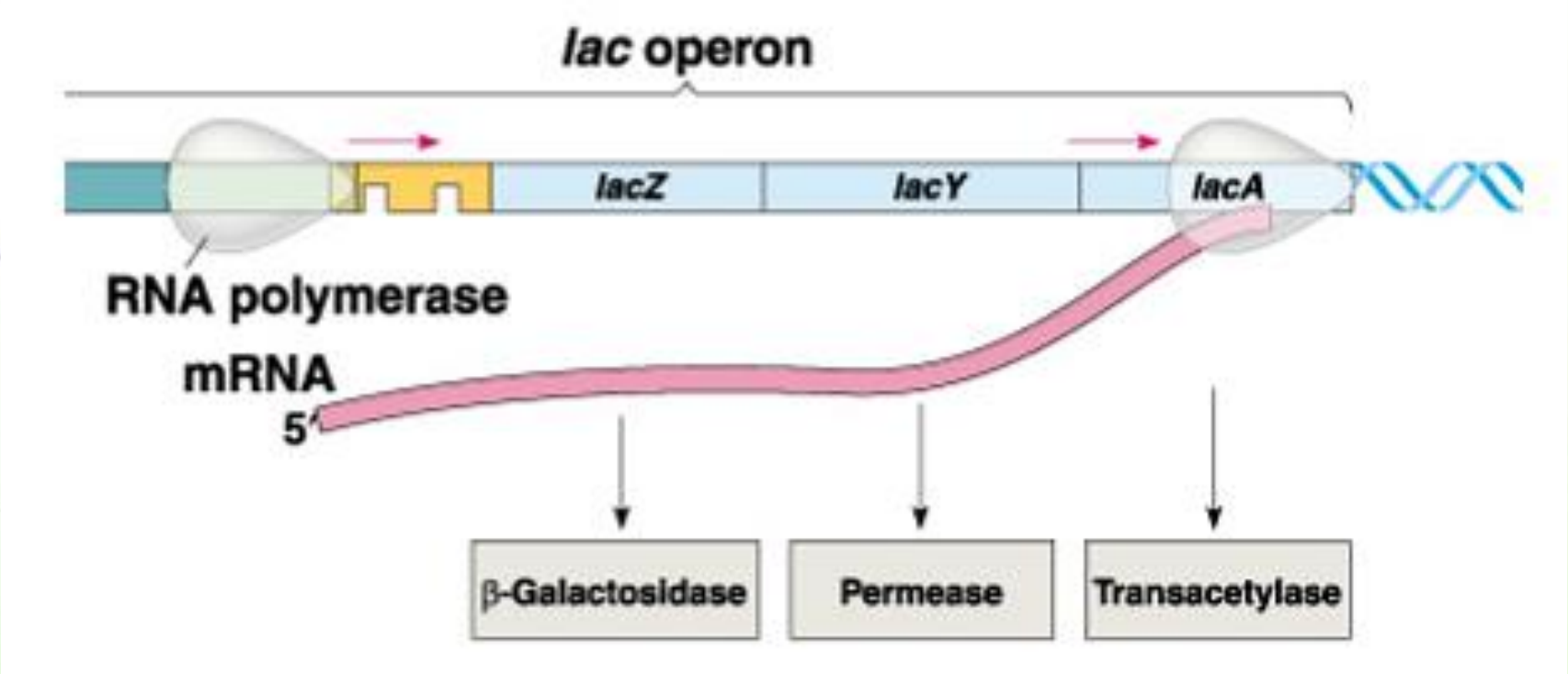


Components of the lac operon



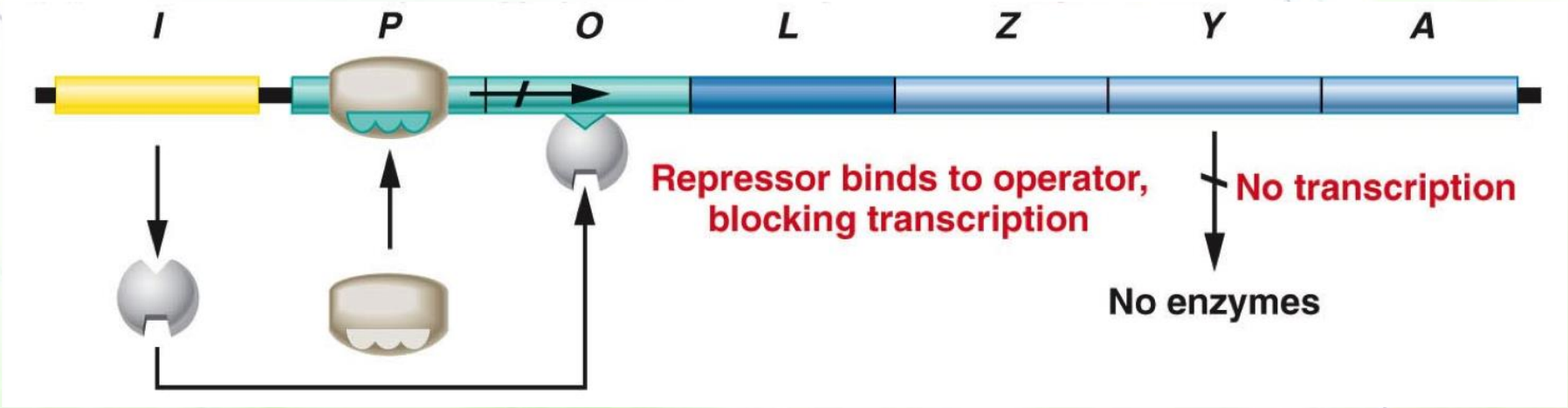
What is an operon?

- A cluster of genes transcribed from one promoter producing a polycistronic mRNA.



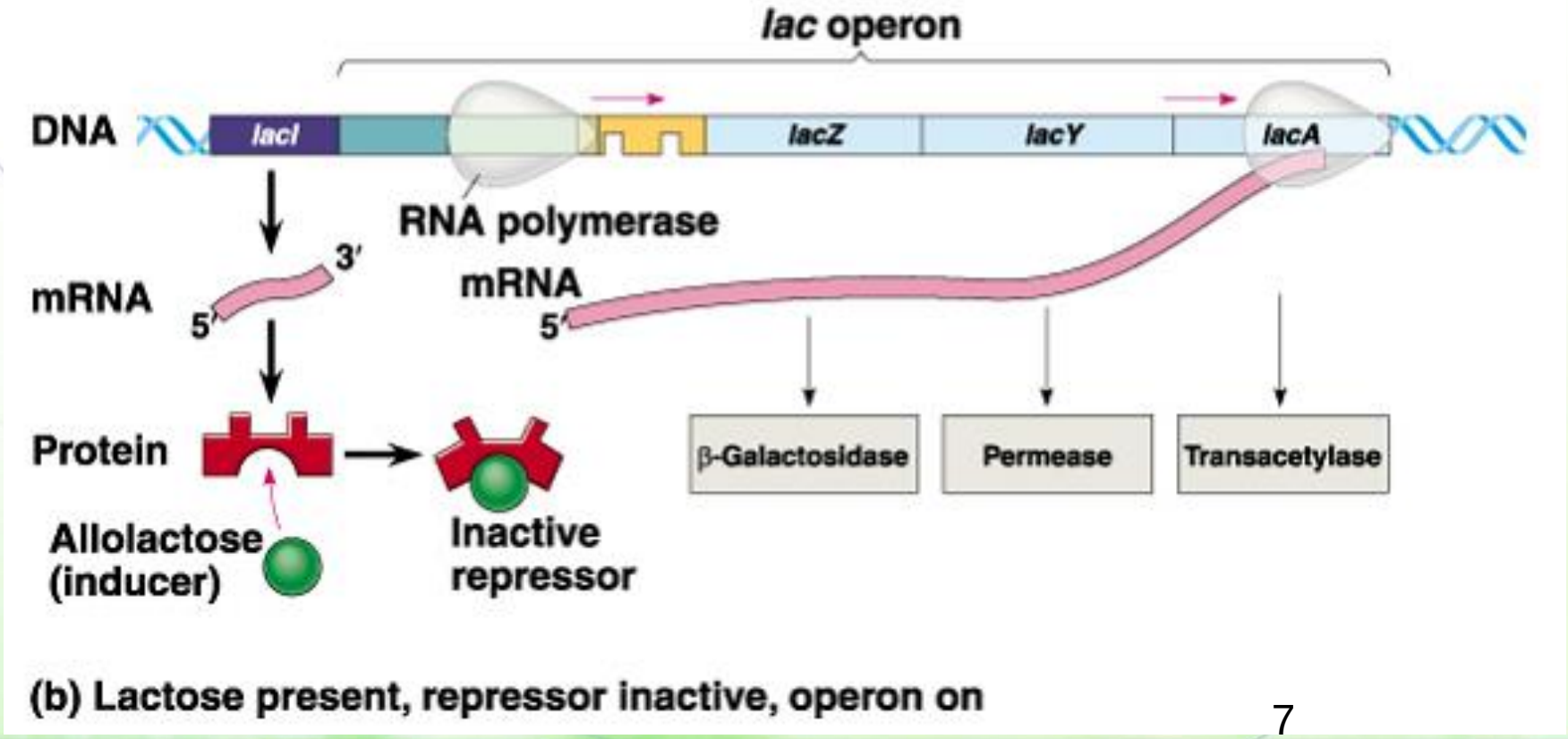
The operator

- The promoter region includes the operator region, which is a binding site of a protein called the lac repressor.
- The lac repressor blocks transcription by preventing the RNA polymerase from binding to the promoter.



Regulation by lactose (positive)

- Lactose binds to the repressor, thereby preventing it from binding to the operator DNA.
- This is known as positive regulation.



(b) Lactose present, repressor inactive, operon on

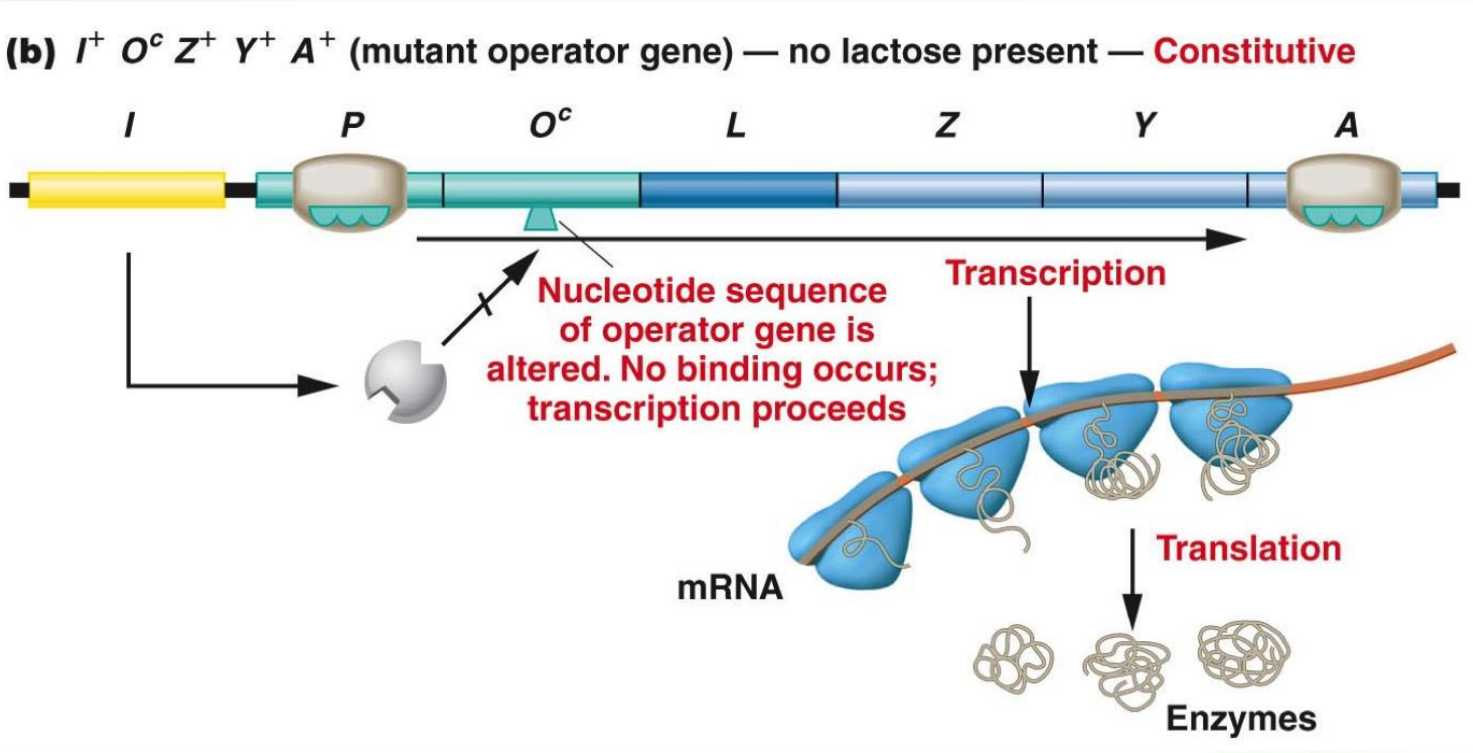
Cis vs. trans regulatory elements

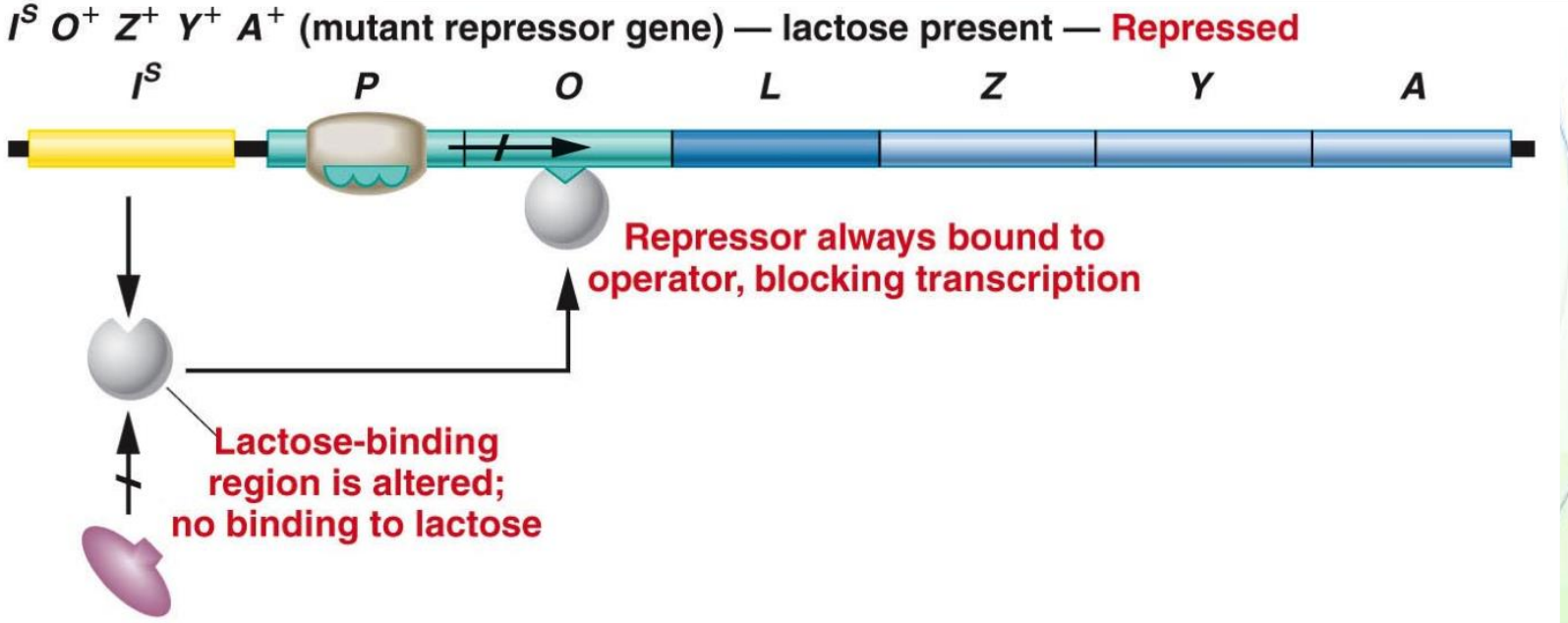
- Regulatory sequences like the operator are called **cis-acting elements** because they affect the expression of only genes linked on the same DNA molecule.
  - Mention other examples of cis-acting elements

- Proteins like the repressor are called **transacting factors** because they can affect the expression of genes located on other chromosomes within the cell. They are produced from **trans-acting elements**.
  - Mention other examples of trans-acting elements

Effect of mutations

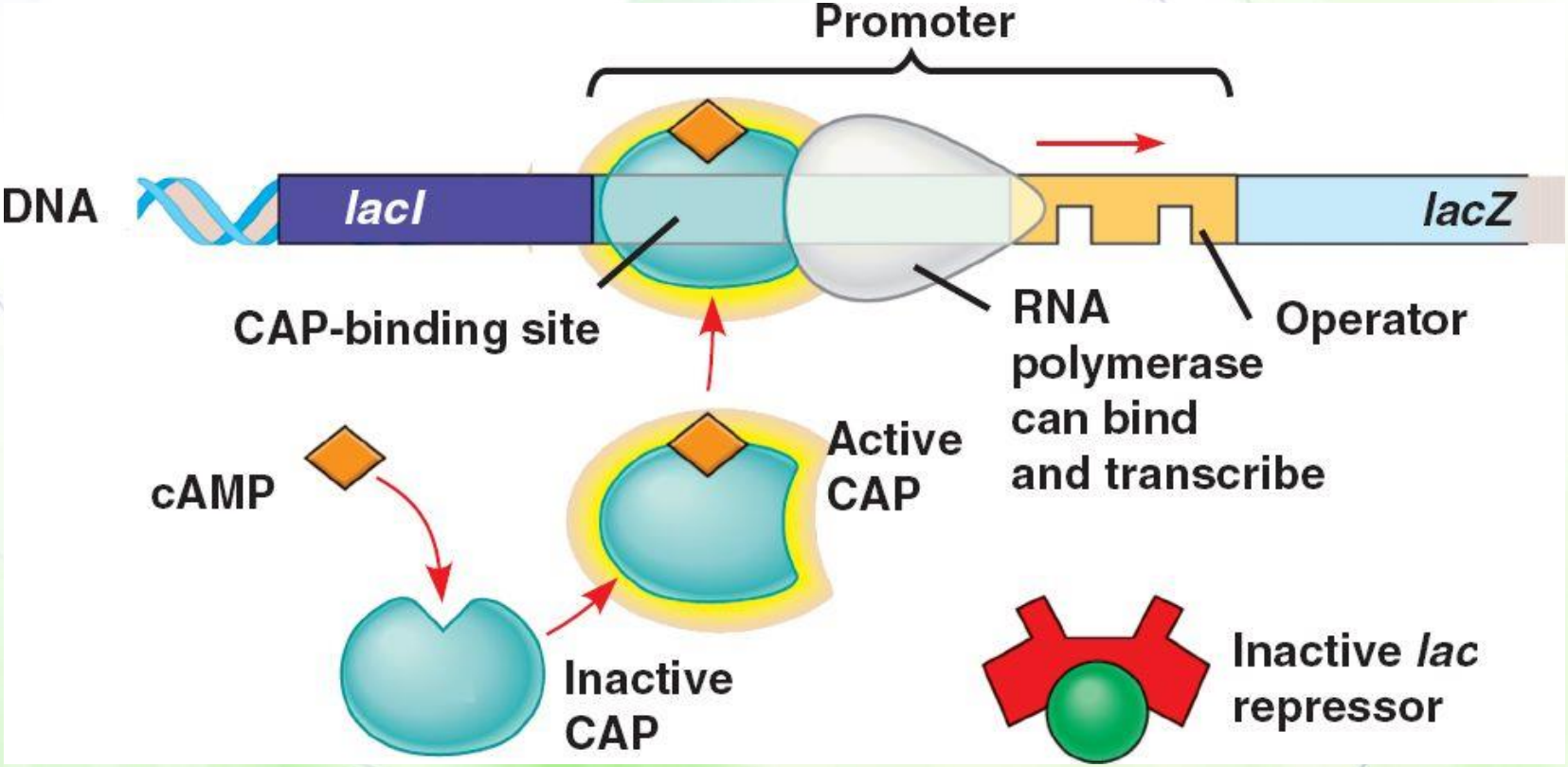
- Some mutations result in **constitutive** expression (always on).
  - Mention examples.
- Other mutations cause **non-inducible** or **repressed** expression (always off).
  - Mention examples


(b) I^+ O^c Z^+ Y^+ A^+ (mutant operator gene) — no lactose present — Constitutive
I
P
O^c
L
Z
Y
A
Nucleotide sequence of operator gene is altered. No binding occurs; transcription proceeds
Transcription
mRNA
Translation
Enzymes


I^S O^+ Z^+ Y^+ A^+ (mutant repressor gene) — lactose present — Repressed
I^S
P
O
L
Z
Y
A
Repressor always bound to operator, blocking transcription
Lactose-binding region is altered; no binding to lactose

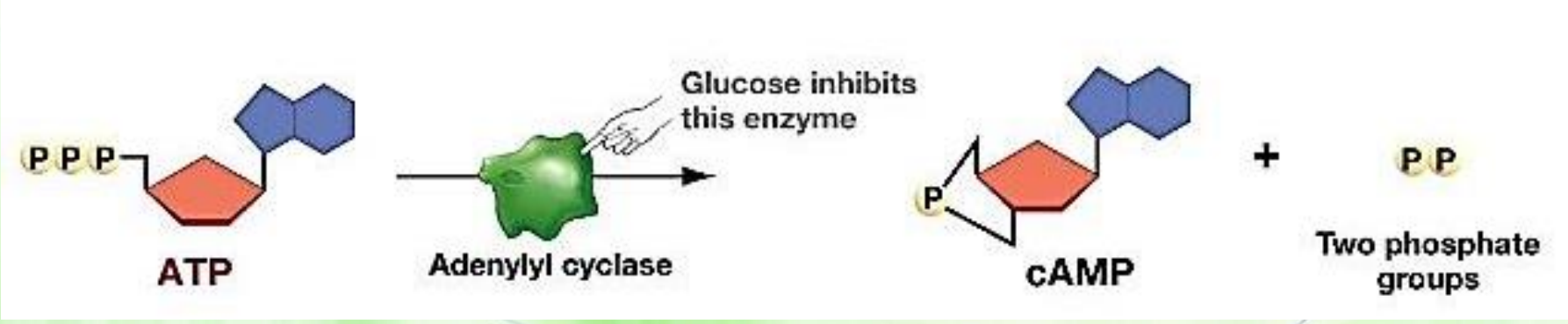
Another level of regulation

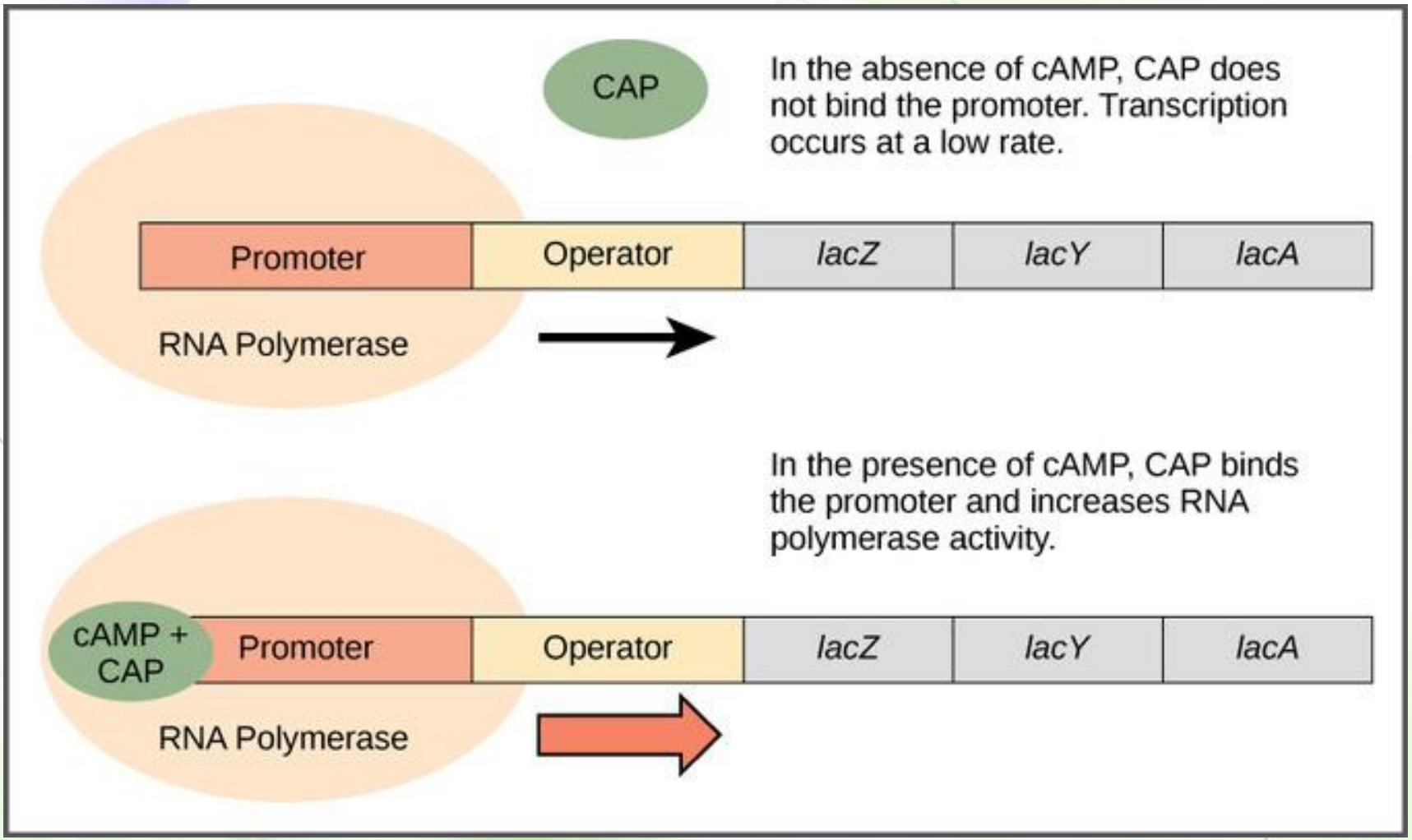
- Another regulator is cAMP, which binds to a protein known as catabolite activating protein (CAP).

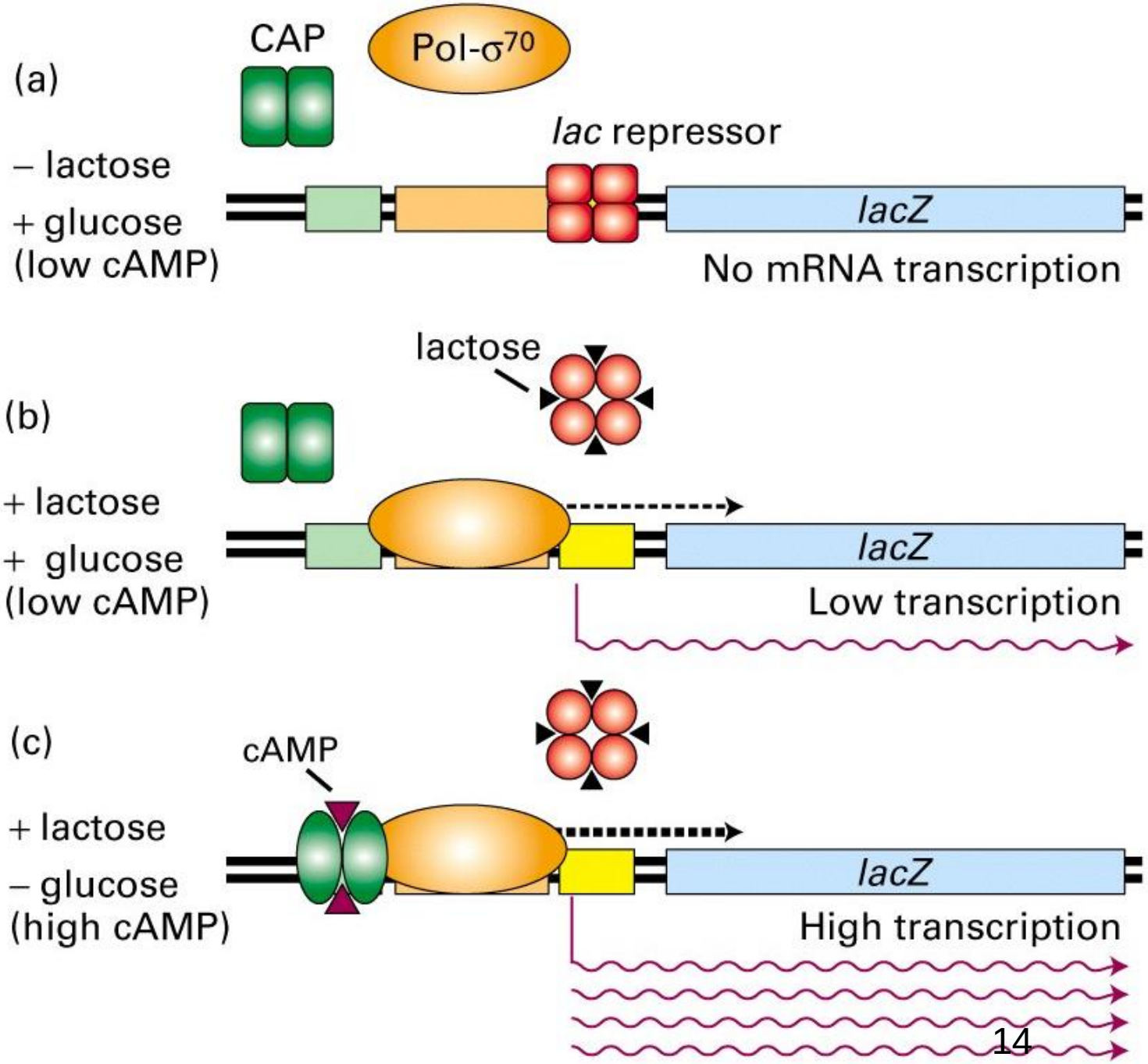


Regulation by glucose (negative)

- The ability of CAP to bind to the promoter is influenced by how much cAMP is in the cell is produced by adenylyl cyclase, which is inhibited by high level of glucose.
- Glucose is preferentially utilized by bacterial cells and it represses the lac operon even in the presence of the normal inducer (lactose).
- This is known as negative regulation.




CAP
In the absence of cAMP, CAP does not bind the promoter. Transcription occurs at a low rate.
Promoter
Operator
lacZ
lacY
lacA
RNA Polymerase
In the presence of cAMP, CAP binds the promoter and increases RNA polymerase activity.
cAMP + CAP
Promoter
Operator
lacZ
lacY
lacA
RNA Polymerase


CAP
Pol-σ70
(a)
lac repressor
– lactose
+ glucose
(low cAMP)
lacZ
No mRNA transcription
lactose
(b)
+ lactose
+ glucose
(low cAMP)
lacZ
Low transcription
(c)
cAMP
+ lactose
– glucose
(high cAMP)
lacZ
High transcription

Regulation of transcription in eukaryotes

Regulatory mechanisms

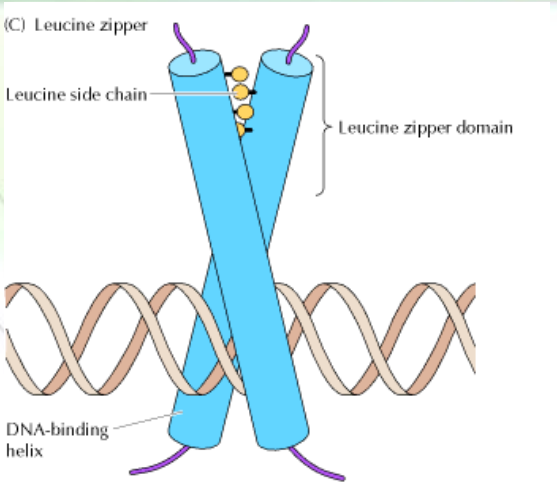
- Although the control of gene expression is far more complex in eukaryotes than in bacteria, the same basic principles apply.
- Transcription in eukaryotic cells is controlled by:
  - Cis-acting elements
    - Promoters, proximal promoter elements, and enhancers
  - Transcriptional regulatory proteins
    - Activators
    - Repressors
  - Chromatin remodeling
  - Noncoding RNA molecules

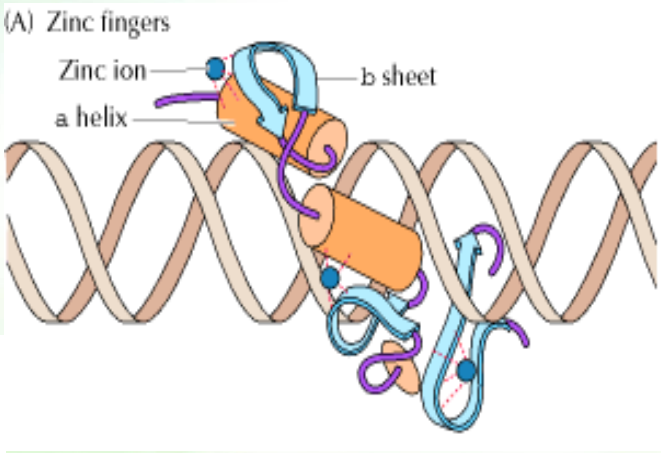
Transcriptional regulatory proteins

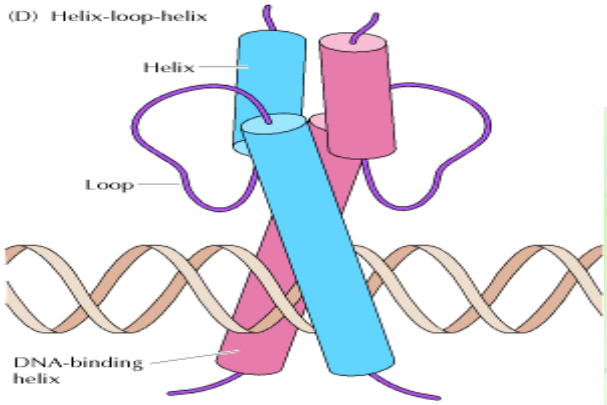
- These proteins consist of *at least* two domains:
  - A DNA-binding domain
  - A regulatory or activation domain that interacts with transcriptional proteins
- Both activities are independent and can be separated from each other

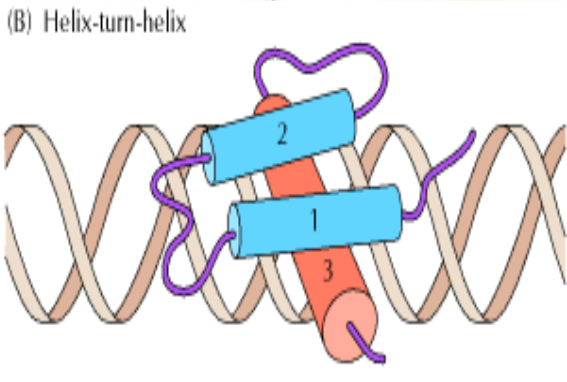
DNA-binding domains

- Zinc finger domains (Steroid receptors)
- Helix-turn-helix motif (homeodomain proteins)
- Leucine zipper (CREB)
- Helix-loop-helix

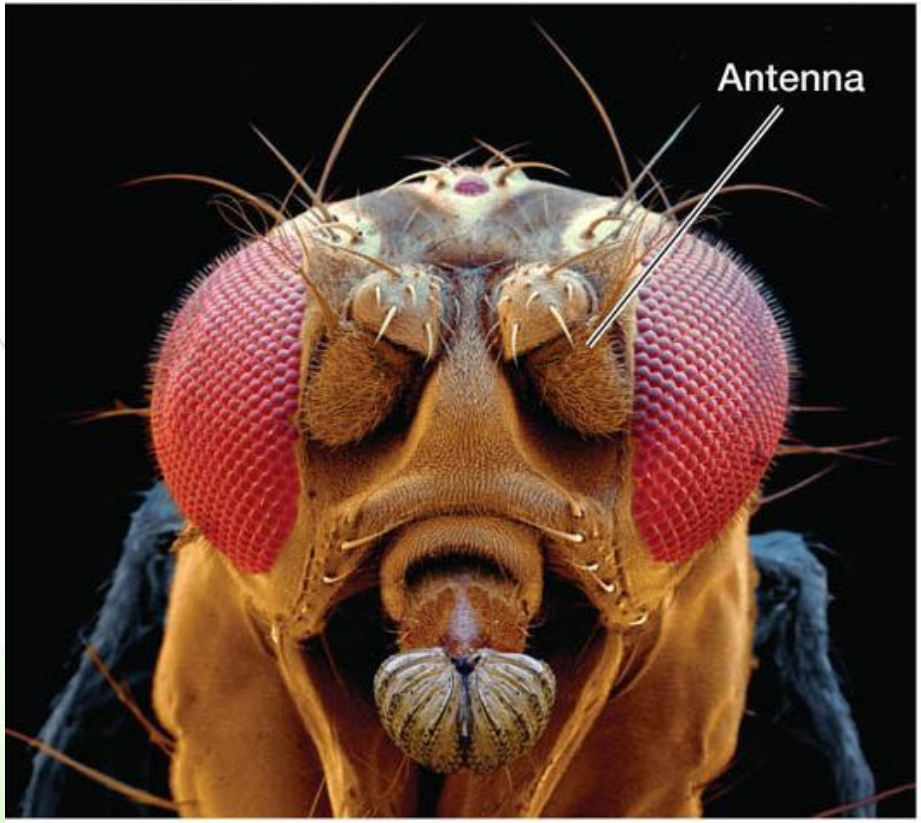








Homeodomain proteins



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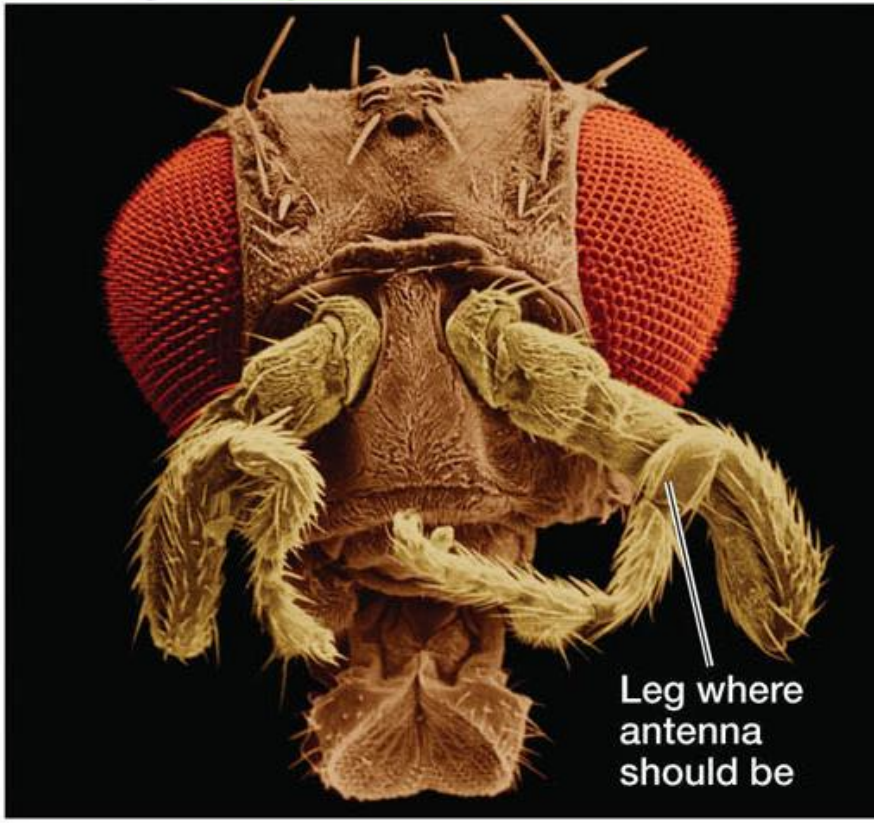

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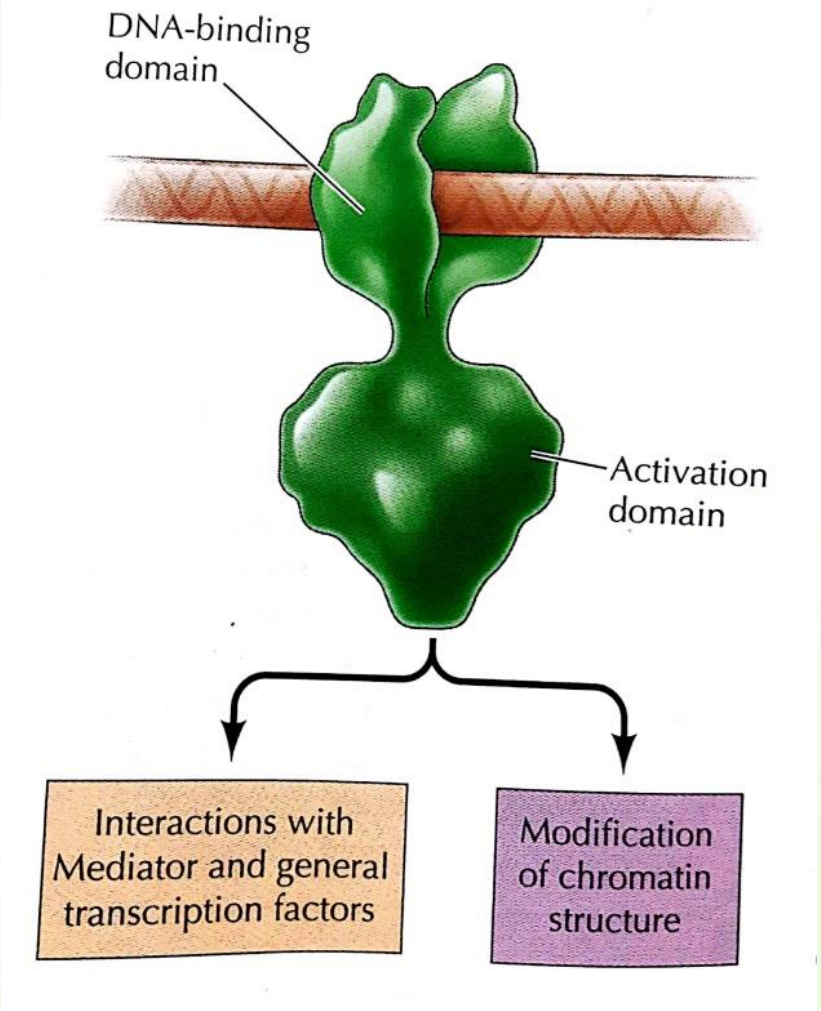
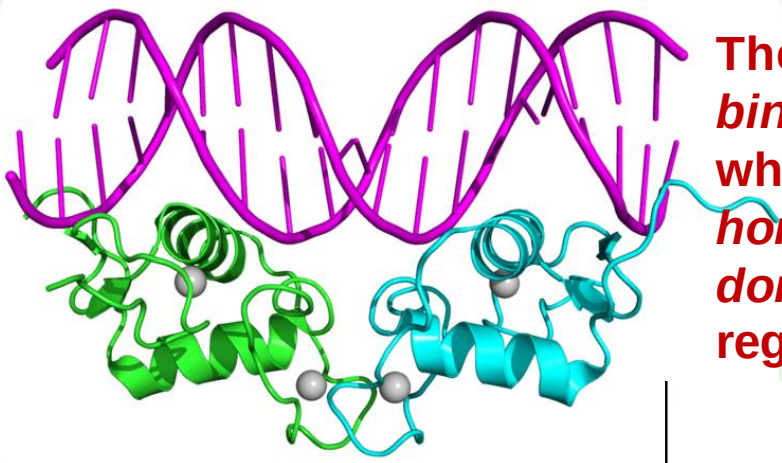
The activation domains

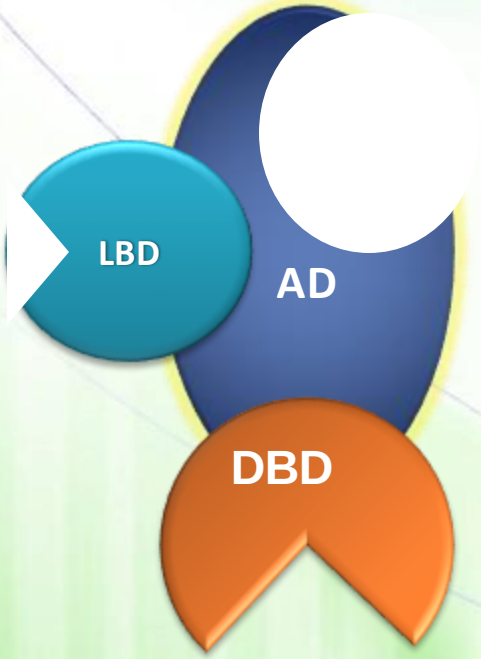
- Activation domains stimulate transcription by
  - interacting with general transcription factors, facilitating the assembly of a transcription complex on the promoter,
  - modifying the chromatin.

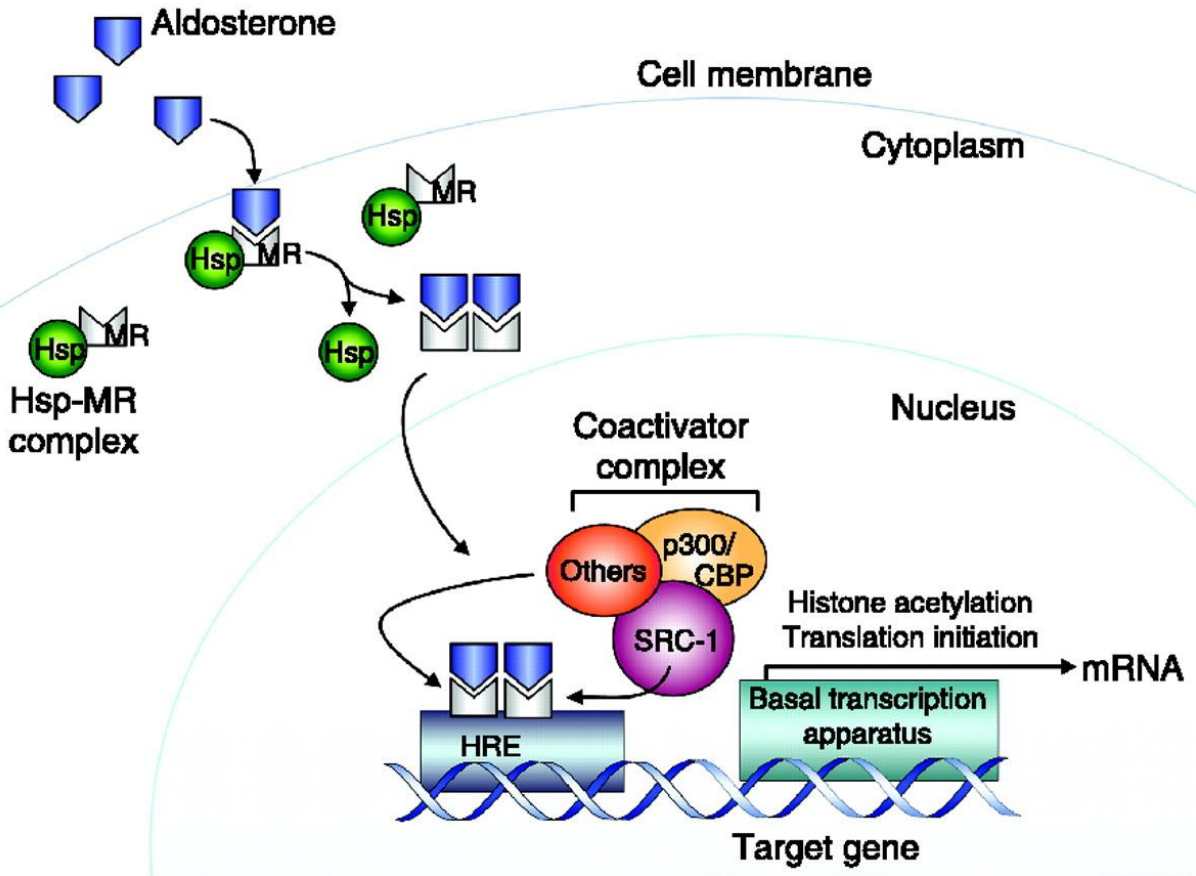


Steroid hormone receptors



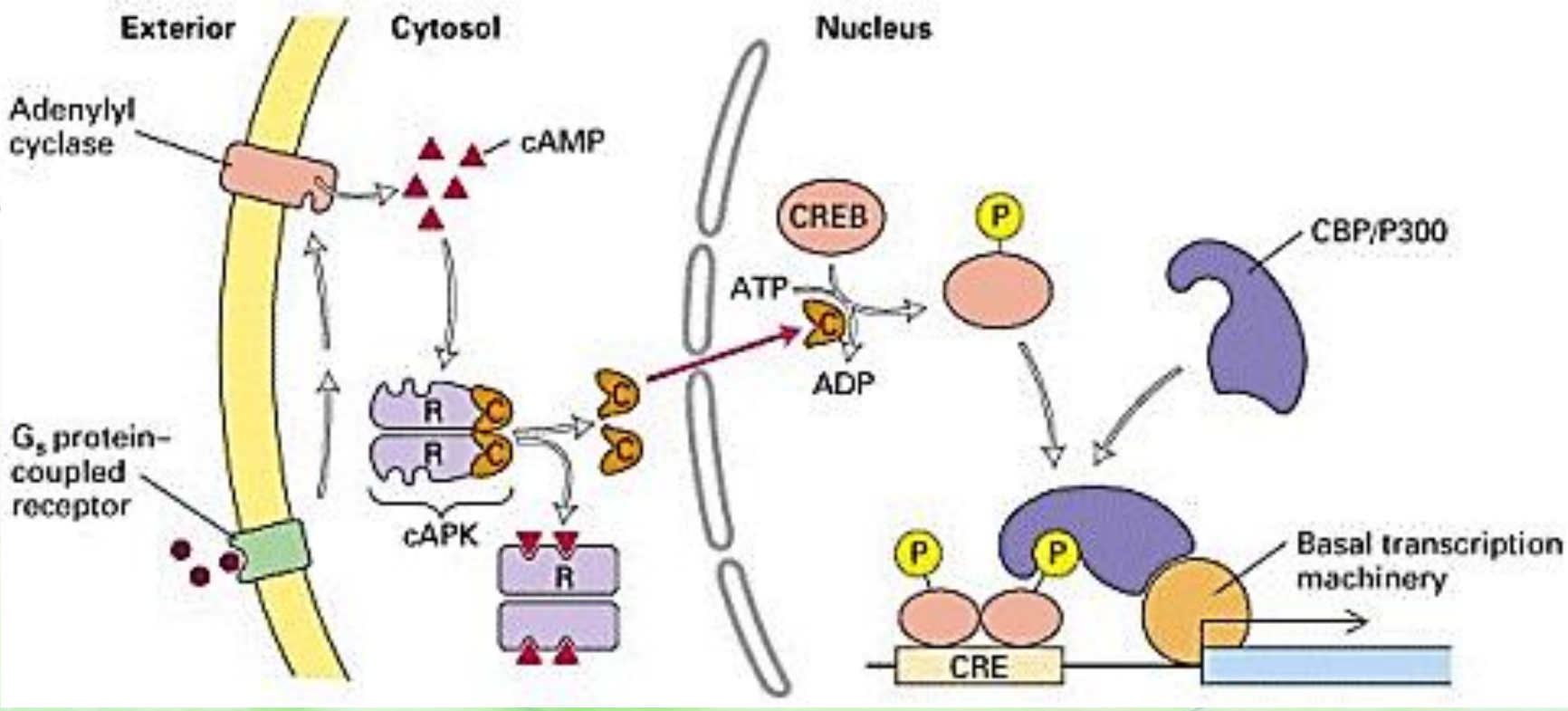
These receptors bind steroid hormones at *ligand-binding domain*, then translocate into the nucleus where they bind specific DNA sequences called *hormone response element* via *their DNA-binding domain*, and recruit and bind transcriptional regulatory proteins using their *activation domain*.





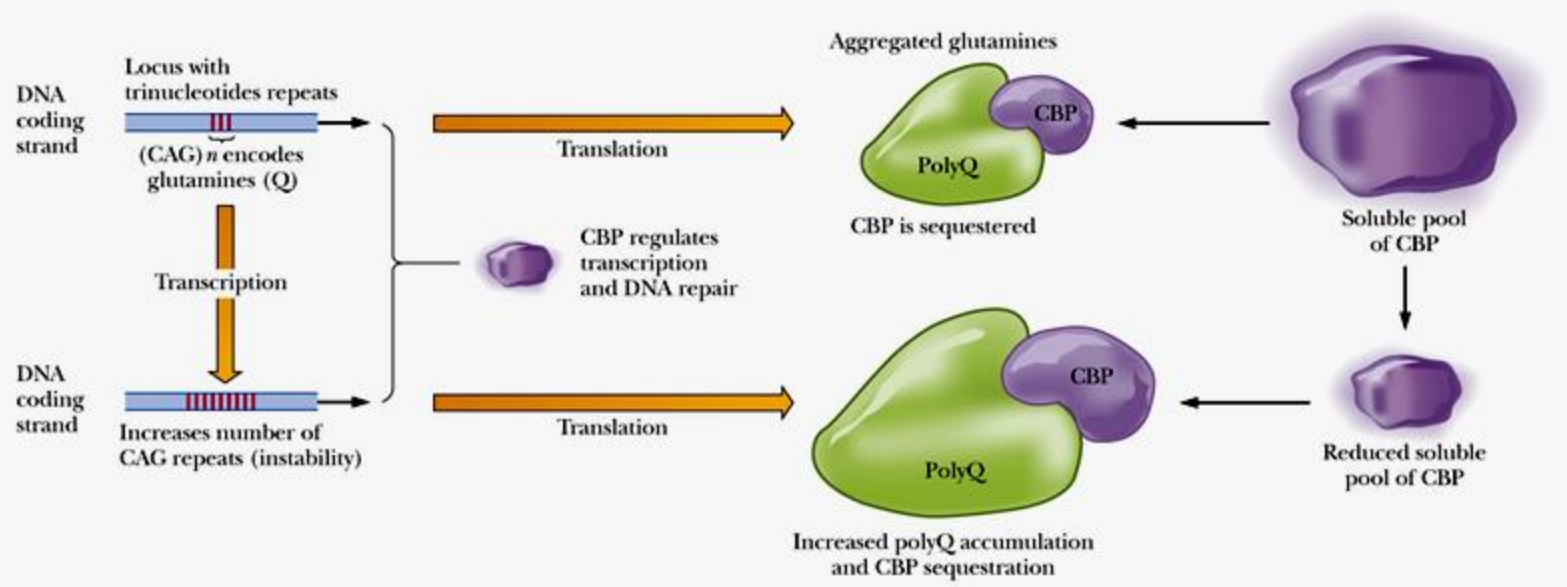
cAMP-response element (CRE) binding protein (CREB)

- In the presence of cAMP, protein kinase A is activated phosphorylating CREB. CREB forms a dimer and binds to CRE. The dimer can then form a complex with the RNA polymerase and, thereby, activating transcription.



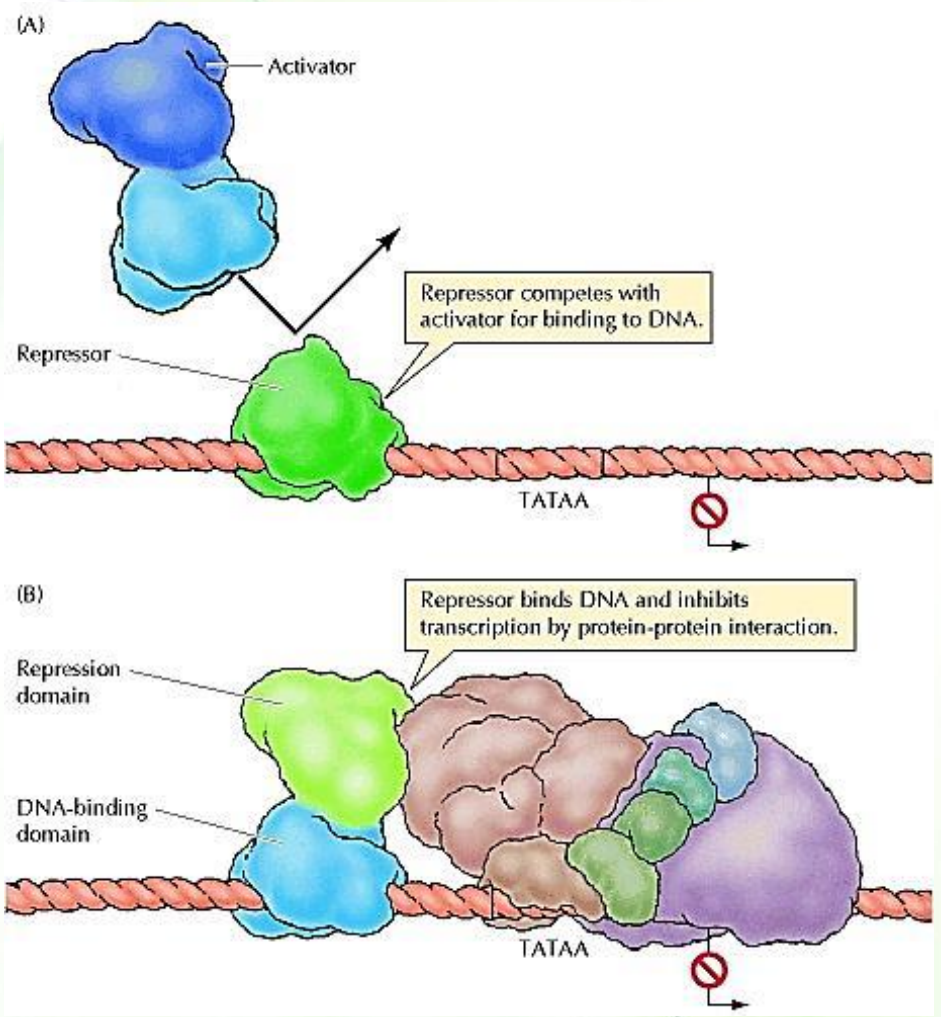
Huntington's disease

- It is a neuro degenerative disease caused by a mutation in the transcription protein, *huntingtin*. The mutation increases the number of CAG repeats, which encodes glutamine.
- The polyglutamine product sequesters CREB-binding protein (CBP), making less of it available for molecular processes, such as transcription and DNA repair leading to cell death.
- The number CAG repeats increases with successive generations, that it becomes worse in successive generations.



Eukaryotic Repressors

- Repressors bind to specific DNA sequences and inhibit transcription.
- Repressors may have
  - both DNA-binding and protein-binding domains
  - DNA-binding domains, but not protein-interaction domains



But really….

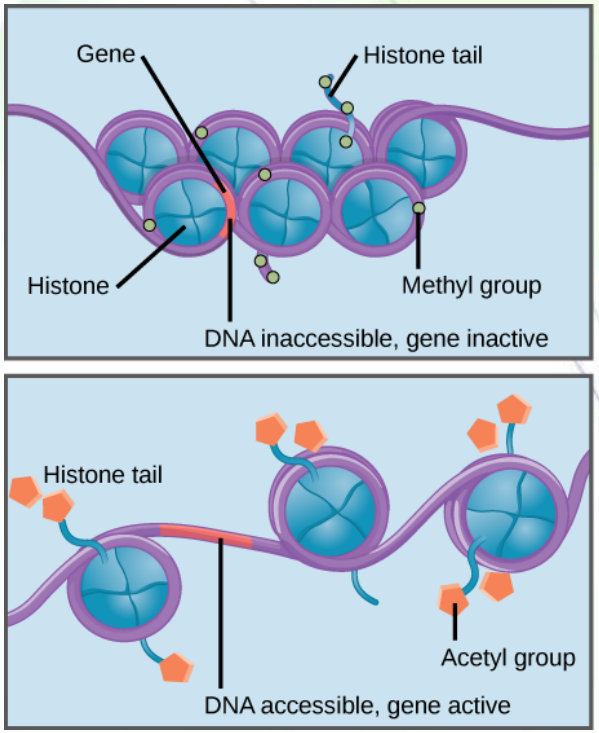
How do TFs regulate gene expression?

- Transcription factors cause epigenetic/e[igenomic changes in DNA.

- What is epigenetics?
- Epi: “above” or “in addition to”
- It indicates genetic alterations in gene expression without a change in DNA sequence.
  - Chromatin packaging
  - Chemical modification of histones
  - Chemical modification of DNA

Modulation of chromosomal structure

- The packaging of eukaryotic DNA in chromatin has important consequences in terms of its availability as a template for transcription
  - Actively transcribed genes are found in loose chromatin (euchromatin)
  - Inactive genes are located in highly packed heterochromatin.

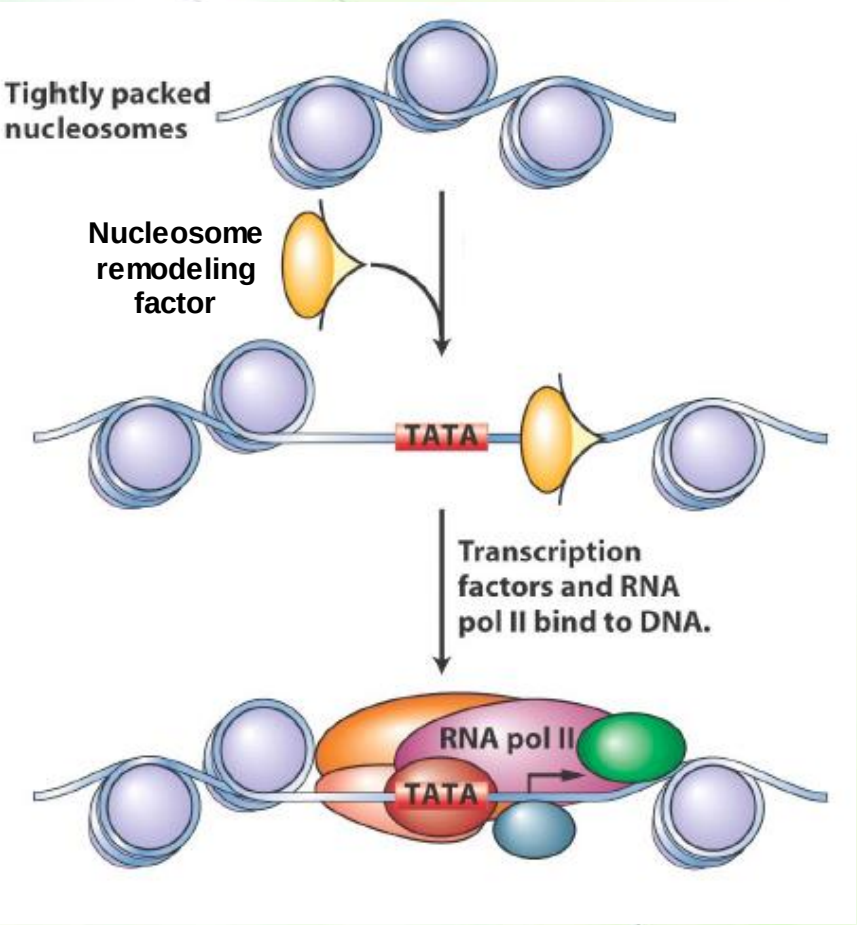


Methylation of DNA and histones causes nucleosomes to pack tightly together. Transcription factors cannot bind the DNA, and genes are not expressed.

Histone acetylation results in loose packing of nucleosomes. Transcription factors can bind the DNA and genes are expressed.

Chromatin remodeling factors

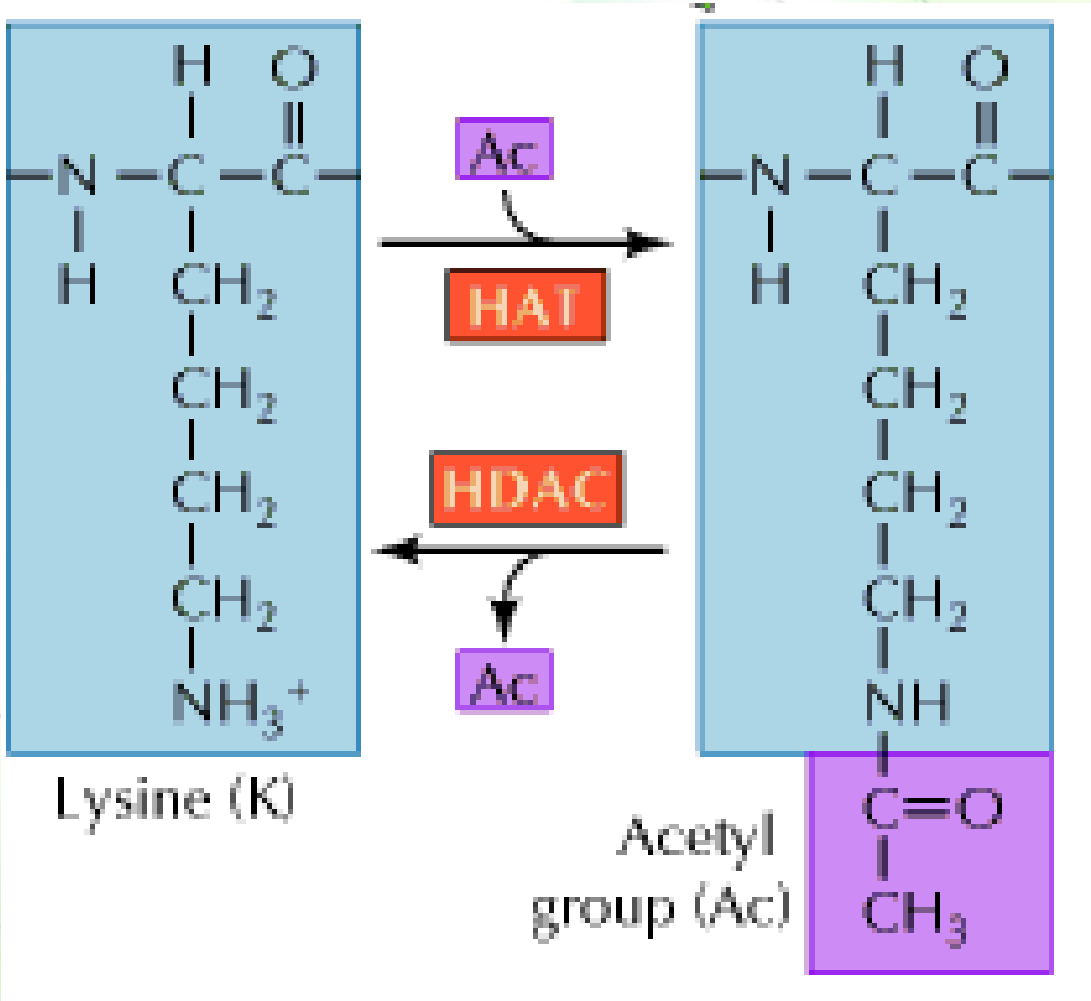
- They facilitate the binding of transcription factors by
  - Repositioning nucleosomes making DNA accessible
  - altering nucleosome structure allowing protein binding to DNA
  - Removing histones from DNA
- Chromatin remodeling factors can be associated with transcriptional activators and repressors.



How else are chromosomal structures altered?

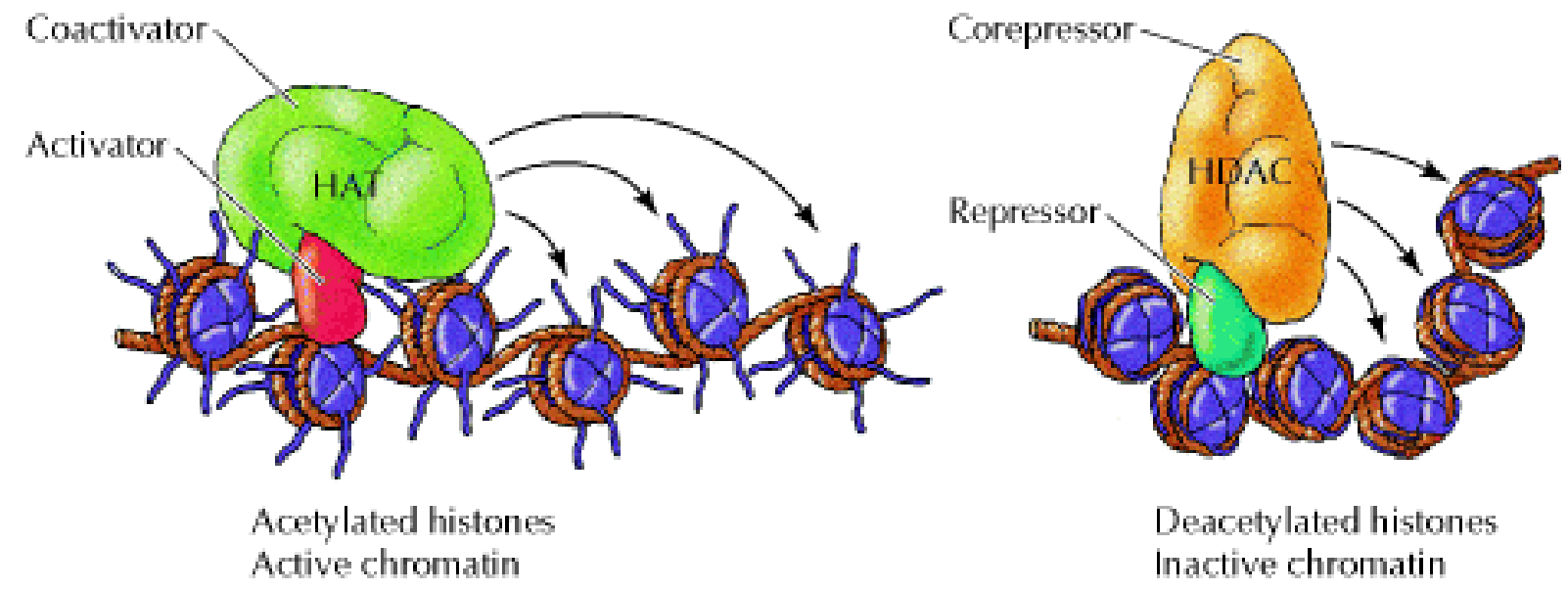
- Change of compactness of the chromatin by:
  - Chemical modification of histones
    - Acetylation, methylation, phosphorylation, and sumoylation (small ubiquitin-related modifier)
  - Binding of noncoding RNAs to DNA

Histone acetylation



Enzymatic association

- Transcriptional activators and repressors are associated with histone acetyltransferases and deacetylases, respectively
  - TFIID associates with histone acetyltransferases.

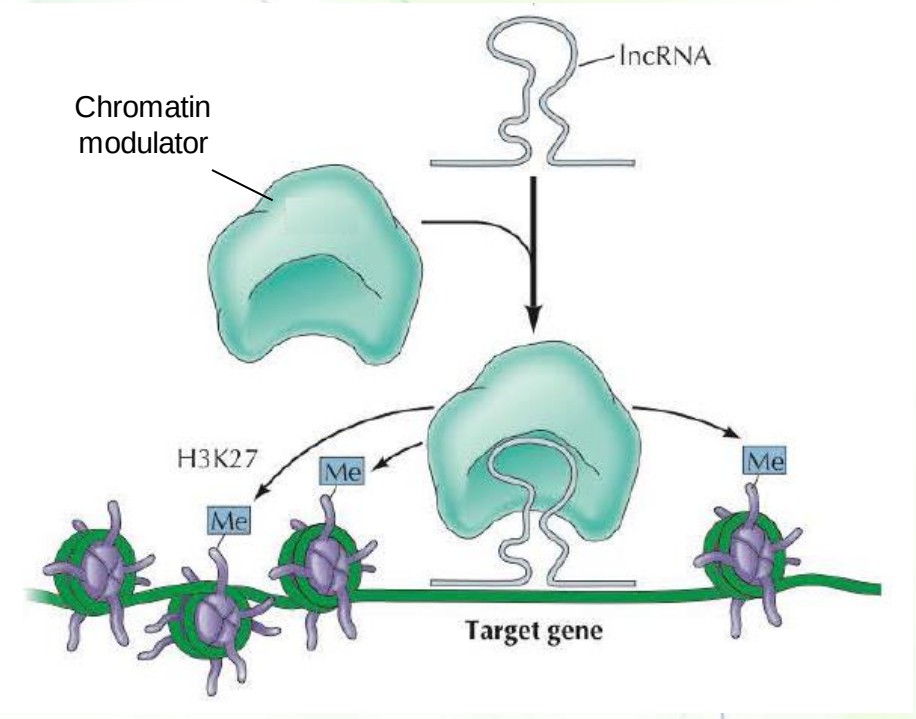


Other modifications of histones

- Histone can also be methylated or phosphorylated.
- Effect is dependent on sites of modification.

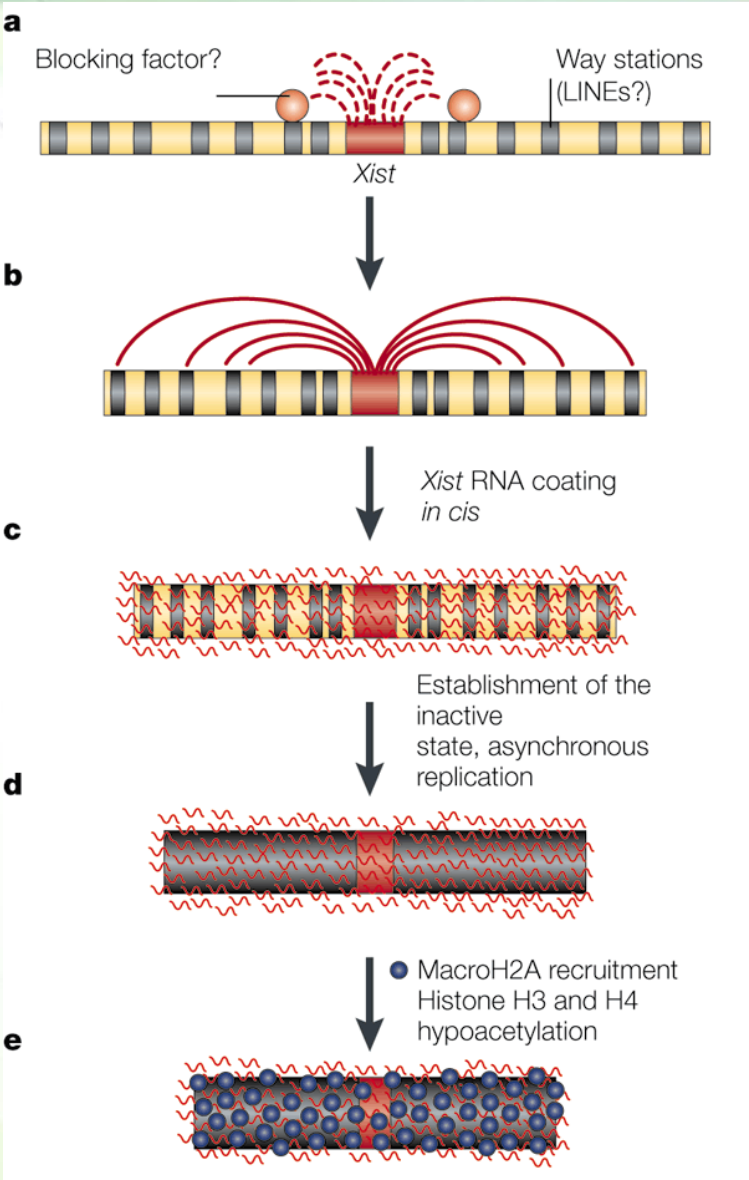
Role of noncoding RNAs

- RNA molecules that are homologous to the DNA or to the growing mRNA sequences of certain genes to induce chromatin condensation and histone methylation.

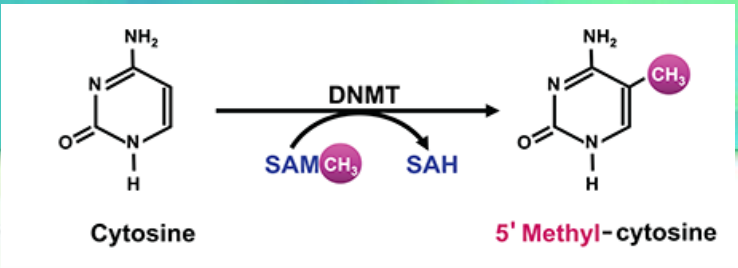


X chromosome inactivation

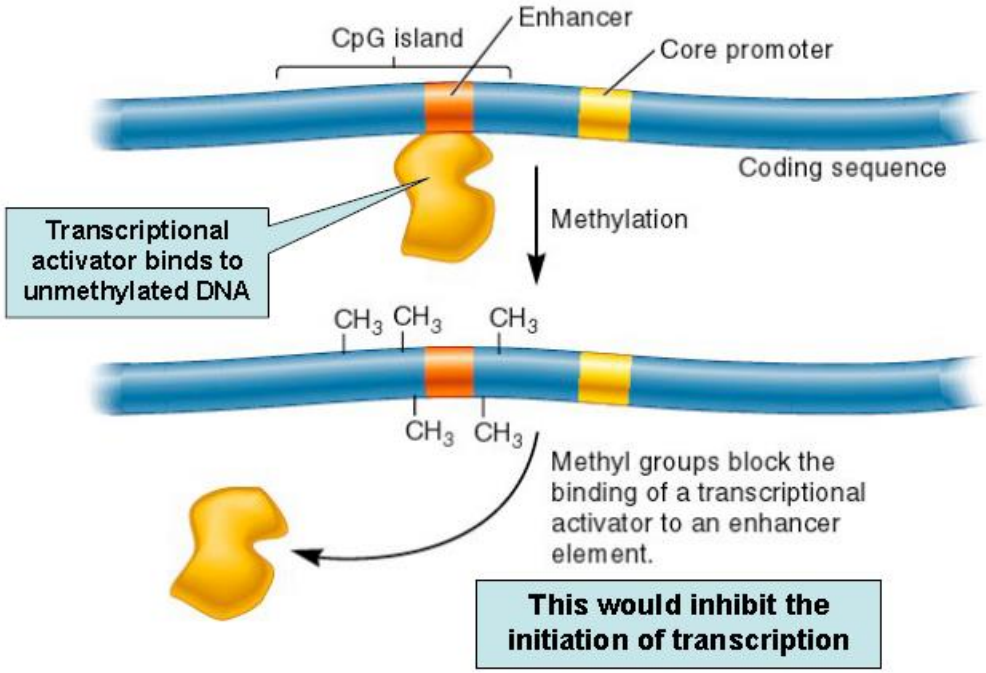
- A long noncoding RNA (lncRNA) is transcribed from *Xist* gene located on the inactive X chromosome.
- The Xist RNA coats the inactive chromosome and promotes the recruitment of a protein complex that methylates histone 3 leading chromosomal condensation.



DNA methylation

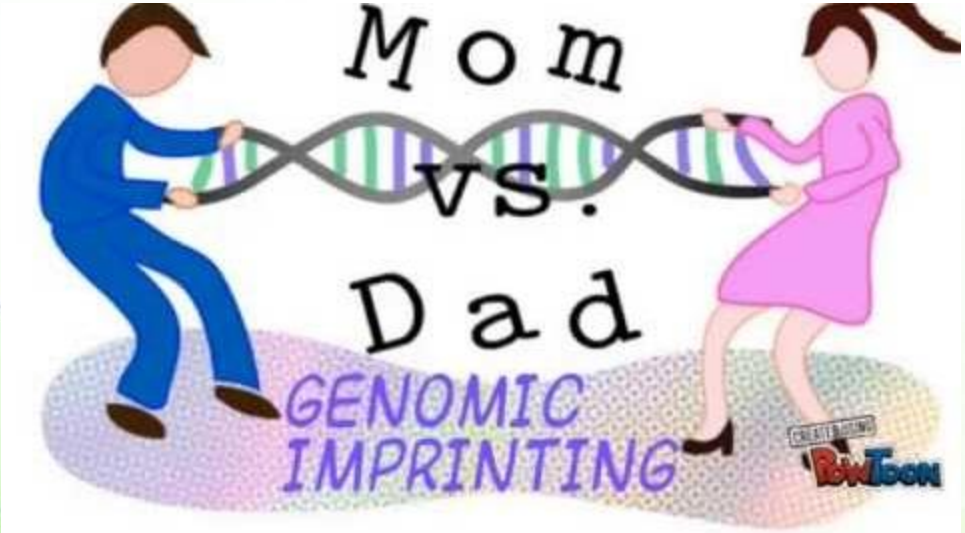


- Cytosine residues can be methylated groups at the 5’-carbon position specifically at CG sequences (called CpG isalnds near promoters).
- DNA methylation reduces gene transcription by blocking of activator binding to DNA and inducing heterochromatin formation.



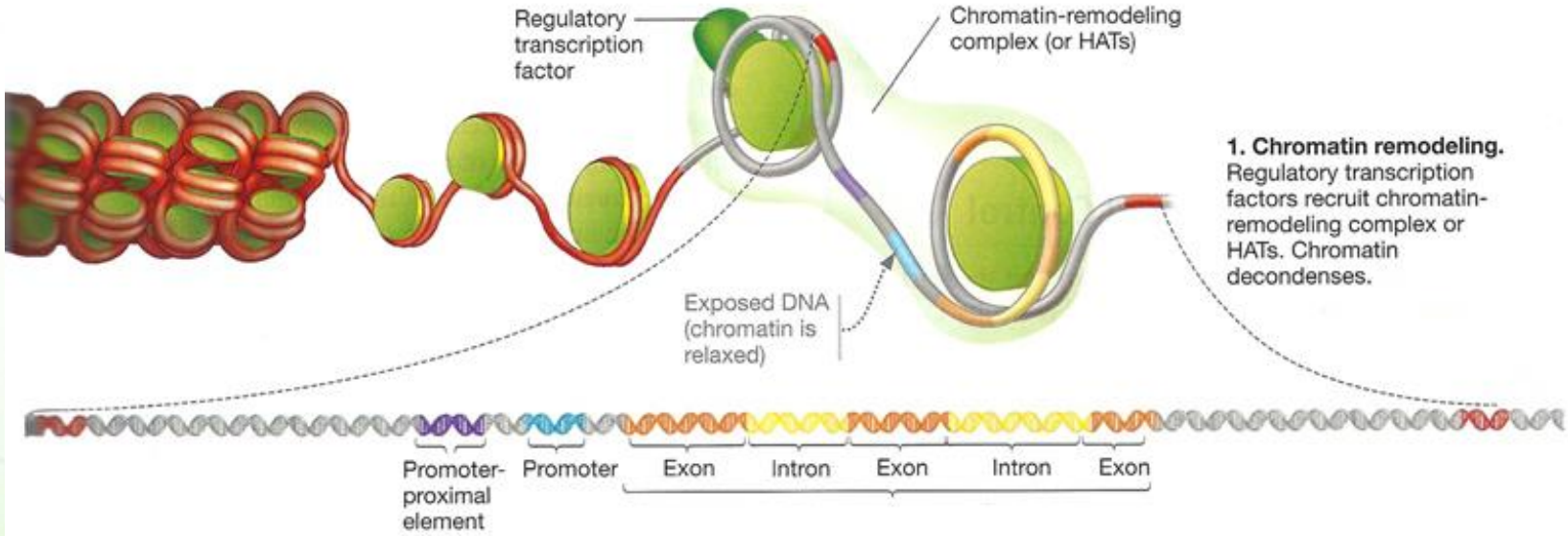
Genetic imprinting

- Methylation is maintained following replication and is inherited.
- Methylation is a mechanism of genomic imprinting (either the paternal gene or the maternal gene is active).

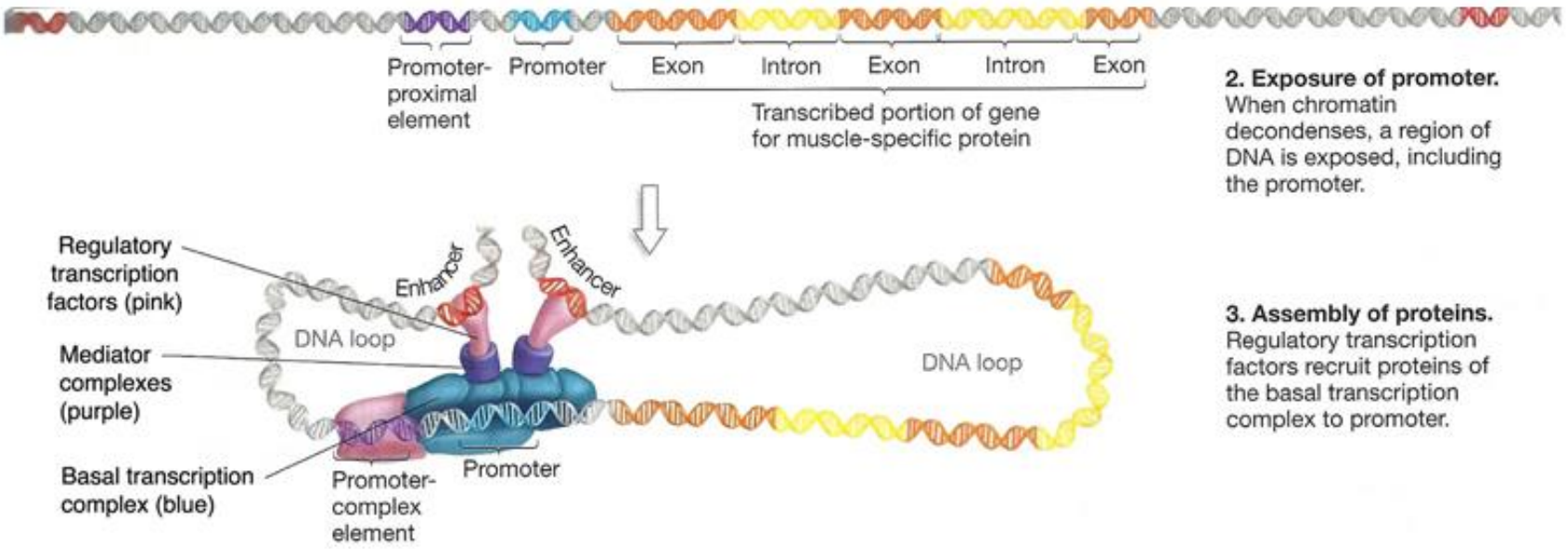


Scenario

Chromatin remodeling exposes the promoter



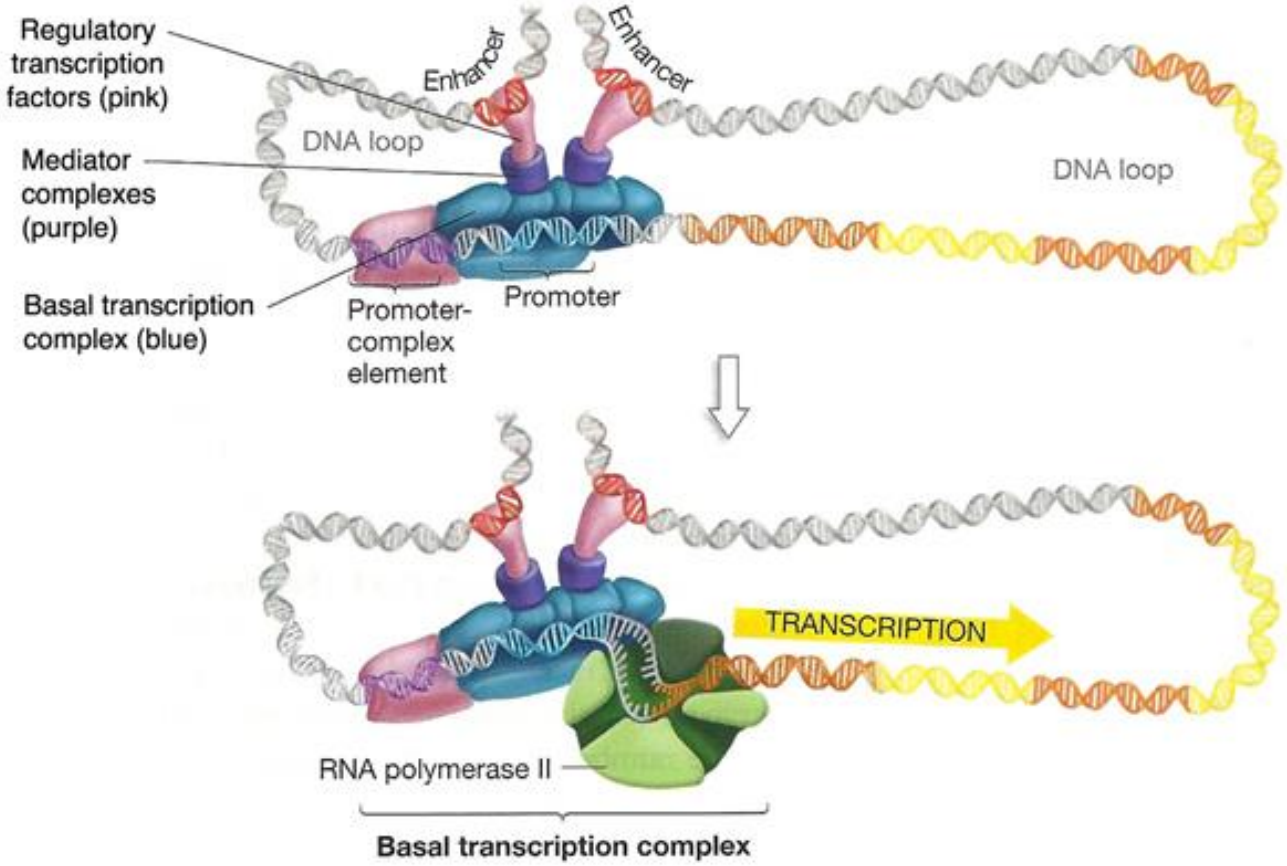
Assembly of basal transcription complex



2. Exposure of promoter. When chromatin decondenses, a region of DNA is exposed, including the promoter.

3. Assembly of proteins. Regulatory transcription factors recruit proteins of the basal transcription complex to promoter.

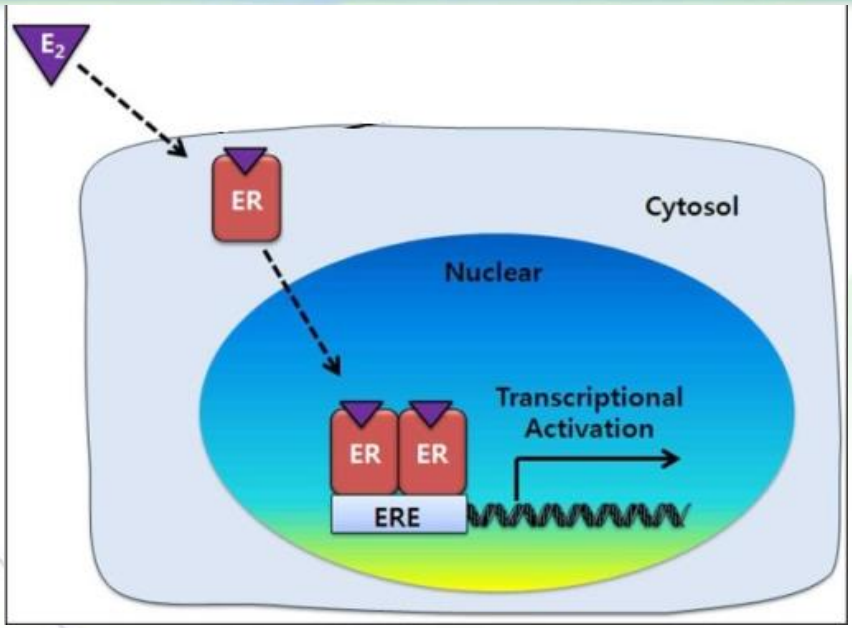
RNA polymerase joins transcription complex

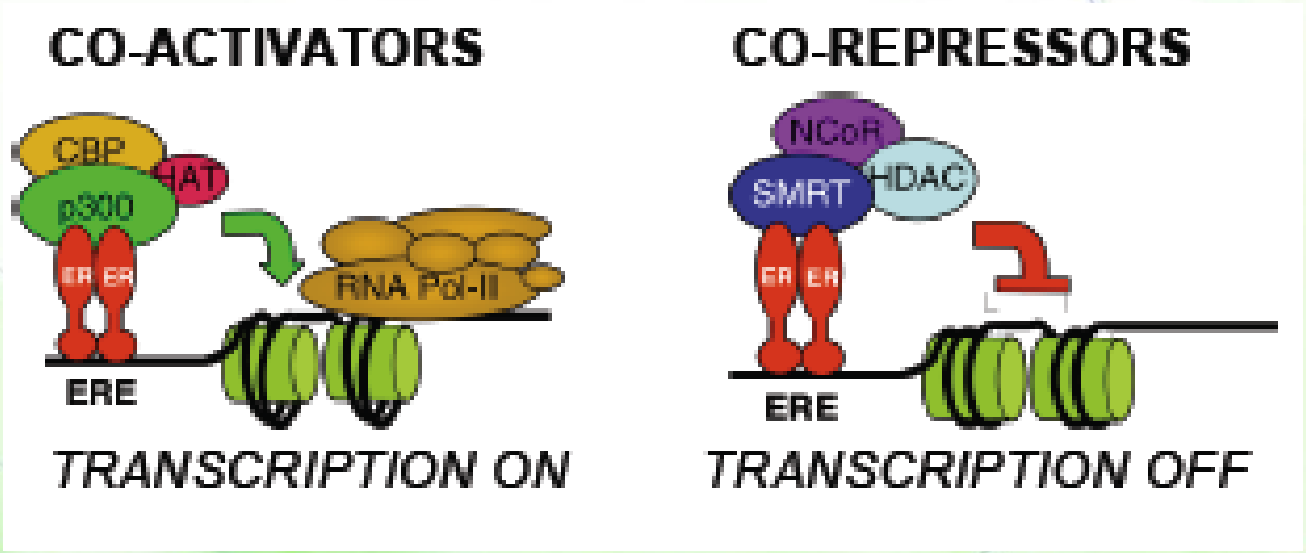


3. Assembly of proteins. Regulatory transcription factors recruit proteins of the basal transcription complex to promoter.

4. Attachment of RNA polymerase. RNA polymerase II completes the basal transcription complex; transcription begins.

Example of steroid nuclear receptors





Identical twins have the exact same genetic information

But their epigenomes become increasingly different over time

- Epigenetic changes can cause dramatic differences between twins, including many cases where one twin develops a disease and the other does not.